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From 85 hereditary breast cancer families, 285 invasive breast carcinomas from patients with known BRCA1/2 mutations were analyzed for histo-pathological features, c-erbB2, estrogen receptor (ER) and progesterone receptor (PR) expression. This study confirms previous data showing that BRCA1 HBC displays an aggressive biological phenotype characterized by high S-phase fraction and low estrogen and progesterone receptor expression suggesting that BRCA1 breast cancer generally will not respond well to therapy with hormone receptor modulators such as tamoxifen. C-erbB2 oncoprotein expression was low, however, which may partially explain why BRCA1 HBC tends to have a prognosis no worse than nHBC. BRCA2 cancers showed a slightly lower average S-phase fraction and higher degree of steroid hormone receptor expression than BRCA1 cancers, but also showed a level of c-erbB2 expression about 2.5 times higher than seen in sporadic breast cancer (48% of cases vs. 16-20% of cases). This suggests a somewhat more aggressive BRCA2 phenotype than previously thought and underscores the importance of c-erbB2 testing in HBC and nHBC.

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INTRODUCTION

Even before the discovery and isolation of the major breast cancer genes *BRCA1* and *BRCA2*, there were clues that the pathology of HBC differed from that of its sporadic counterpart.[1] Earlier work showed 1) more medullary carcinoma -- a proliferative, high-grade special type with good prognosis -- in familial and HBC settings[2-5]; 2) a higher mitotic grade in the no special type (NST, or ductal) invasive carcinoma in HBC[1,3]; 3) a statistically insignificant association of invasive lobular[6-8] or tubular[9,10] carcinomas in familial settings; and 4) conflicting positive[11,12] and negative[6] associations of lobular carcinoma *in situ* (LCIS) with family history. Only one of these, a positive association, was statistically significant.[1,11]

BODY

As of the end of this study 285 cases from 85 families with a BRCA1 or BRCA2 mutation were identified and used for the following analysis.

The BRCA1 HBC Phenotype

At Creighton University, the authors have been investigating the clinical and pathological features of HBC with one of the largest and most longstanding pathology resources. By 1994, sufficient linkage information on *BRCA1* had become available to assign the high mitotic grade of the NST carcinomas in the cohort[3] to the *BRCA1*-related HBC subset.[1,13-16] This result has been confirmed in all subsequent studies of *BRCA1* HBCs (Table 1).[17-20] The early claim that the high mitotic grade segregates with mutations in the two terminus regions of the gene[21] has not been confirmed by other groups,[20,22,23] and we do not see such an effect in the Creighton data set

(J.N.M., unpublished data). In addition to mitotic grade, tubular and nuclear grades also are increased in *BRCA1* HBC (Table 1).

The excess of medullary and atypical medullary carcinomas, which had long been observed in the Creighton HBC cases,[4] was assigned by the authors to the *BRCA1* subset shortly after the gene was isolated.[13,15,16] This result was confirmed by the Breast Cancer Linkage Consortium[17] and other groups (Table 1).[24,25] Like HBC, medullary carcinoma is more common in early onset breast cancer,[1] but the excess of medullary and atypical medullary carcinoma in *BRCA1* HBC is independent of this age covariate.[13,17] Compared with sporadic breast cancers[15] or with *BRCA2* HBC (Table 2), *BRCA1* HBC shows significantly more of a superfamily of types which we term "medullary group" carcinomas (medullary, atypical medullary, and NST with medullary features). These have in common a heavy infiltration of lymphocytes and plasma cells in the tumor stroma. The Breast Cancer Linkage Consortium has confirmed that stromal mononuclear cell infiltration is a feature of *BRCA1* HBC.[19]

BRCA1 HBC shows a deficit of *in situ* carcinoma compared with non-HBC (Table 1).[13,17] The prevalence of *BRCA1* and *BRCA2* mutations in women under age 50 is much lower for those with DCIS (13%) than invasive breast cancer (24%) ($p = 0.0007$).[26] We have also found a deficit in low grade special type carcinomas which we call "tubular-lobular group," or TLG, carcinomas, comprised of lobular, tubulolobular, tubular, and invasive cribiform types (Tables 1 and 2). These special types were considered together (see Marcus et al.[16] and references therein) because they share certain histological features (including the hybrid type, tubulolobular), secretory properties, epidemiological risk factors, an association with family history in earlier reports, and in the case of lobular carcinoma, a virtual lack of somatic alteration at the 17q *BRCA1* site and more microsatellite instability, in contrast to ductal carcinomas.

As further evidence for a proliferative phenotype, the authors found that a high DNA S-phase fraction originally observed in the whole HBC cohort[27] was confined to the *BRCA1* subset.[1,13,15] Jóhannsson et al.[22] have confirmed this observation (Table 1). The Creighton data also showed that *BRCA1* HBC is more prevalently aneuploid than non-HBC, and that the average aneuploid DNA index is less compared with non-HBC (Table 1).[13,15,16] p53 tumor suppressor protein is more frequently increased in *BRCA1* breast cancers compared with sporadic breast cancers.[20,28-30] This had been observed earlier in the Creighton breast-ovary HBC family cases,[31] most of which were later identified as *BRCA1* HBC. Overexpression of p53, almost always the result of a mutation in the *TP53*gene, has been observed in general in highly proliferating breast carcinomas.[32] The *TP53* mutations in *BRCA1* and *BRCA2* breast cancers are unusual in that that most of them are not in usual "hotspots," and are distributed in a region of the protein on the opposite side of its DNA-binding surface.[33]

Jóhannsson et al.[22] showed that *BRCA1* HBCs have decreased expression of estrogen receptor, progesterone receptor, and *c-erb-B2* (Her2/*neu*) oncoprotein (Table 1). These results have been confirmed by the Breast Cancer Linkage Consortium[29] and by us (N. Lehman, unpublished data).

All of the evidence thus indicates a remarkable proliferative phenotype in *BRCA1* breast cancers, and high proliferation rates are a characteristic of the ovarian carcinomas in the Creighton families with breast-ovary (and mostly *BRCA1*) HBC as well.[34] The distinctive pathobiology of *BRCA1* HBC can be understood in context with a model of tumor genetic evolution. In the model,[35,36] intermediate and transformed cells suffer small losses or gains of chromosomal material while remaining near diploid in DNA content (DNA index = DI ~1). At some point the chromosome complement endoreduplicates to near-tetraploidy (DI ~ 2), with

continuing and more severe segmental or complete chromosomal loss, which progressively lessens the DI. In this scenario, hyperdiploid breast cancers with $1.3 \leq DI \leq 1.7$, higher S phase fractions, and p53 mutations are among the most "evolved."[37-39] The *BRCA1* phenotype – aneuploidy, lower aneuploid DI (see Table 2), high proliferation, and p53 overexpression – fits the profile of a genetically evolved tumor. In order to evolve genetically, the target cell must proliferate. In the model, the *BRCA1* mutation would put the intermediate target cell[40,41] on a fast track of increased or unregulated proliferation, beginning near the time of menarche. At transformation, the intrinsic high proliferation rate is locked in to the tumor as a fossil phenotype of the intermediate cell.[42] The BRCA1 protein fulfills the role required in this model. When mutated, its well-established antiproliferative function[43-45] is lost, which may send the target cells into unregulated proliferation.

The BRCA2 HBC Phenotype

The *BRCA2* HBC phenotype is less well determined than that of *BRCA1* HBC because there are fewer cases and probably greater intrinsic heterogeneity, leading to less concordance in the literature. Most studies agree that the age of onset is significantly greater than in *BRCA1* HBC (Table 2), but still considerably less than in non-HBC. All studies agree that there is a lesser propensity for the NST (ductal) carcinomas to form tubules (Table 2), as in *BRCA1* HBC. The pathologists in the blinded Creighton studies[13,15,16] (J.N.M. and D. L. Page) have made special efforts to not underdiagnose the tubular-lobular group (TLG) carcinomas. They find that TLG group carcinoma is a powerful discriminator between *BRCA1* and *BRCA2* HBC, scarce in the former but prevalent in the latter (Table 2). Consistent with this result, Armes et al.[24] also find

increased lobular carcinoma in *BRCA2* HBC, but other groups do not,[17,46] for reasons that may relate to differing diagnostic thresholds or to intrinsic differences in the data sets.

The Breast Cancer Linkage Consortium[17] claims a higher grade for *BRCA2* HBC than for the age-matched non-HBC controls, but this result is questionable. Higher nuclear, mitotic, and total grades are not seen in the large Creighton data set displayed in Table 2. The problem is that the Consortium *BRCA2* pathology data set is dominated by the Icelandic founder 999del5 mutation, which comprises nearly half (49%) of its cases. The pathology associated with this mutation, reported in a separate publication by non-Consortium pathologists,[46] is remarkable for very high grades which do not appear typical of the non-999del5 *BRCA2* cases in other data sets. Because the Consortium cases are so heavily weighted with this specific mutation, its overall results may be skewed toward higher grade. Thus, despite its large size, the Consortium data base may not be representative of *BRCA2* HBC at large. On the other hand, the fact that this argument can be made is itself evidence that *BRCA2* HBC phenotype, at least insofar as the 999del5 mutation is concerned, is heterogeneous.

In the Creighton *BRCA2* HBC data set, there is prevalent lobular neoplasia, defined as lobular carcinoma in situ or atypical lobular hyperplasia. In one *BRCA2* HBC family, 10 of 13 invasive breast carcinomas were associated with lobular neoplasia. Of interest, TLG carcinomas have a high prevalence of lobular neoplasia (see Marcus et al.[16] and references therein). The DNA cytometric characteristics of *BRCA2* HBC differ from those of *BRCA1* HBC (Table 2): there is lesser aneuploidy and a lower mean aneuploid S phase fraction, which is more in line with the characteristics of non-HBC (Table 2). Estrogen receptor, progesterone receptor, p53 and Her2/*neu* proteins also appear to be expressed at levels comparable to those in non-HBC (Table 1).[20,22,28-31,47-49]

KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES

- 285 cases ascertained for analysis
- 152 risk factor questionnaires have been completed
- 187 tumors analyzed by Dr. Joseph Marcus and Dr. David Page
- 98 additional cases to be added to Drs. Marcus and Page's analysis
- 70 cases have completed ER, PR, c-erB-2 analysis

CONCLUSION & DISCUSSION

Clinical Implications of *BRCA1* and *BRCA2* HBC Pathophenotypes

The decreased estrogen and progesterone receptor expression suggests that *BRCA1* breast cancer generally will not respond well to therapy with hormone receptor modulators such as tamoxifen. However, we have cautioned[50] that this supposition should not preclude consideration of such therapy in chemoprevention trials, for there is no evidence that the pre-transformed target intermediate cell lacks receptors. Early data in the National Surgical Adjuvant Breast and Bowel Project prevention trial (NSABP-P1) indicate that tamoxifen may not reduce breast cancer incidence among *BRCA1* mutation carriers, but may reduce the incidence among *BRCA2* mutation carriers, whose tumors are usually estrogen receptor-positive when they develop (Table 1).[51] However, the numbers of positive events in the trial currently is quite small and the results do not reach statistical significance for either *BRCA1* or *BRCA2* carriers. Clearly this and other trials with larger numbers of *BRCA1* and *BRCA2* mutation carriers and more extended follow-up times are needed before this issue can be properly addressed.

The prognosis of *BRCA1* HBC has been a matter of ongoing debate. The issue is important because it weighs in the decisions on prophylactic therapies. Most studies find no significant differences in survival in comparison with non-HBC,[15,49,50,52-54] but better[55] and worse[56,57] outcomes have also been reported. Methodologic differences may account in part for the variability in results.[58] Why the prognosis of *BRCA1*-related HBC, with its adverse pathology markers, would be no worse than non-HBC is a conundrum that is deepened by the observation that in the Creighton families, *BRCA1*-related HBC cases fare better than non-*BRCA1*-related HBC cases, which have neutral pathology indicators.[15,50] But there are clues that *BRCA1* HBC is not an ordinary high grade breast cancer. As we have seen, it does not highly express *c-erbB2*, a marker of poor prognosis,[22,29] and a high proportion are medullary carcinomas,[13,15,16,17,24,25] which in pure forms are prognostically favorable, despite their high mitotic and nuclear grades.[59] Might genetic instability in *BRCA1* HBC – manifest by the prevalent aneuploidy, low aneuploid DNA index, and increased p53 expression described above – indicate fragility and increased susceptibility to chemotherapy and radiation therapy? These observations and questions point to directions for future investigation.

Less is known about the prognosis of *BRCA2* HBC. The best available evidence from several studies is that survivals probably do not differ from non-HBC when adjusted for other variables such as stage.[60]

The pathobiologic features of *BRCA1* and *BRCA2* HBC summarized in Tables 1 and 2 offer some clues as to whether a patient in a family untested for the genes may lie in one or the other syndrome. However, these should not be regarded as sufficiently sensitive and specific to serve as a substitute for syndrome identification by direct genetic testing for germline mutations. Recently, a small number of *BRCA1* HBC, *BRCA2* HBC, and non-HBC tumors were looked at

for expression of 5361 genes by microarray technology.[61] The analysis disclosed 176 genes that were differentially expressed in tumors with *BRCA1* vs. *BRCA2* mutations, and that the expression profiles could accurately classify the tumor as having arisen from a germline mutation in *BRCA1* or *BRCA2*. If these results can be confirmed with larger numbers of cases, this technology could become a powerful tool in diagnosing *BRCA1* or *BRCA2* mutation carrier status from the breast tumors directly.

Please see Appendix A for updated analysis on ER/PR/ c-erB-2 expression in BRCA1/2 breast cancer tumors.

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Table 1: Pathobiologic Characteristics of BRCA1 and BRCA2 Compared With Non-Hereditary Breast Cancers: Literature Summary			
Characteristic	BRCA1	BRCA2	References
Histologic type			
Medullary and atypical medullary	↑	↔?	Marcus et al (1996, 1997a, 1997b); Breast Cancer Linkage Consortium (1997); Armes et al (1998); Eisinger et al (1998)
Lobular and variants	↓	↑?	BRCA1: Marcus (1997a); BRCA2: Armes et al (1998a)
Tubular-lobular group	↓	↑?	Marcus et al (1996, 1997a)
Any DCIS	↓	↔	Marcus et al (1996); Breast Cancer Linkage Consortium (1997)
Any LCIS	↓	↔?	Marcus et al (1996); Breast Cancer Linkage Consortium (1997)
Histologic grade			
Total	↑	↔?	Marcus et al (1996, 1997a,b); Eisinger et al 1996; Breast Cancer Linkage Consortium (1997); Johannsson et al (1997); Armes et al (1998a); Lynch et al (1998); Noguchi et al (1999)
Tubular	↑	↑	BRCA1: Marcus et al (1996, 1997a); Eisinger et al (1996); BRCA1 and BRCA2: Breast Cancer Linkage Consortium (1997); Lakhani et al (1998)
Nuclear	↑	↔?	BRCA1: Eisinger et al (1996); BRCA1 and BRCA2: Breast Cancer Linkage Consortium (1997)
Mitotic	↑	↔?	Marcus et al (1996, 1997a, 1997b); Eisinger et al (1996); Breast Cancer Linkage Consortium (1997); Lynch et al (1998); Lakhani et al (1998)
DNA cytometry			
Diploidy	↓	↔	Marcus et al (1996, 1997a, 1997b); Johannsson et al (1997)
Non-diploid S-phase fraction	↑	↔	Marcus et al (1996, 1997a, 1997b; Johannsson et al (1997)
Protein markers			
Estrogen receptor	↓	↔	Johannsson et al (1997); Karp et al (1997); Verhoog et al (1998); Lynch et al (1998); Loman et al (1998); Noguchi et al (1999); Stoppa-Lyonnet et al (2000); Lakhani et al (2002)
Progesterone receptor	↓	↔	Johannsson et al (1997); Verhoog et al (1998); Lynch et al (1998); Loman et al (1998); Stoppa-Lyonnet et al (2000); Lakhani et al (2002)
p53	↑	↔	Thor et al (1992); Armes et al (1998b); Lynch et al (1998); Noguchi et al (1999); Lakhani et al (2002)
c-erbB2 (Her2/*neu*)	↓	↔	Johannsson et al (1997); Noguchi et al (1999); Lakhani et al (2002)

Symbols in columns 2 and 3 indicate increased (↑), decreased (↓), or similar (↔) incidence of a characteristic as compared with non-hereditary breast cancer. "?" in column 3 indicates non-unanimity in the literature and evidence for possible heterogeneity in *BRCA2* HBC

Table 2: BRCA1 vs. BRCA2 Hereditary Breast Cancer: The Creighton University Series[1]			
	BRCA1	BRCA2	p[2]
Number of families with pathology assessment	29	10	
Number of invasive carcinomas in females	108[3]	37[3]	
Clinical Features			
Age of onset (mean ± SD) (yr)	42.9 ± 12.6	49.1 ± 12.3	0.011
Bilateral cases	34 (31.5%)	8 (21.6%)	0.30
Male cases (excluded)	2	2	
Tumor size (mean ± SD) (cm)	2.1 ± 1.3	1.8 ± 1.2	0.25
Lymph node-positive cases	30 (31.9%)	12 (50.0%)	0.15
Pathologic Types and Features			
Medullary group (medullary, atypical medullary, ductal with medullary features)	43 (40.6%)	4 (12.5%)	0.003
Tubular-lobular group (lobular, tubulolobular, tubular, cribiform special types and variants)	14 (13.2%)	15 (46.9%)	0.0001
Any ductal carcinoma in situ (DCIS)	31 (28.7%)	20 (54.0%)	0.009
Any lobular carcinoma in situ (LCIS)	2 (1.9%)	5 (13.5%)	0.012
Any lobular neoplasia (LCIS + atypical lobular hyperplasia)	3 (2.5%)	11 (29.7%)	<0.0001
Mononuclear cell infiltration absent	3 (9.4%)	9 (27.5%)	0.019
Pathologic Grades			
Mitotic grade 3	43 (53.1%)	6 (25.0%)	0.020
Nuclear grade 3	45 (55.1%)	2 (8.3%)	<0.0005
Tubular grade 3	72 (88.9%)	19 (79.2%)	0.30
Final grade 3	39 (56.5%)	4 (21.1%)	0.009
DNA Cytometry			
Diploid	10 (14.9%)	10 (50.0%)	0.002
Non-diploid	57 (85.1%)	10 (50.0%)	
S-phase fraction, diploids (mean ± SD, %)	2.78 ± 1.73 n = 10	3.53 ± 1.73 n = 10	0.48
S-phase fraction, non-diploids (mean ± SD, %)	15.77 ± 6.82 n = 56	7.36 ± 4.87 n = 10	<0.0005

1) Fisher's exact test for 2 × 2 tables or 2-tailed Student t-test for means and standard deviations.

2) The numbers of cases simultaneously reviewed by the project pathologists (J. N. Marcus and D. L. Page). The denominators implicit from the percent figures that follow are sometimes less than these totals if not every tumor was available for assessment of a given feature.

Appendix A

Pathobiology of BRCA1 and BRCA2 Hereditary Breast Cancers: Update of c-erbB2 Expression, Steroid Hormone Receptor Expression, and DNA Ploidy and S-phase Analysis Studies.

Introduction

Most cases of breast cancer are sporadic. Hereditary breast cancer (HBC) accounts for about 5 to 10% of all breast cancers. Of these, loss of function mutations of the BRCA1 and BRCA2 tumor-suppressor genes are found in the majority of HBC cases. Decreased BRCA1 expression may also occur in some sporadic tumors (Wilson et al., 1999). Study of HBC has led to valuable insight into the pathobiology of breast cancer associated with loss of function of these genes. Both BRCA1 and BRCA2 HBC present at an earlier mean age than non-hereditary breast cancer (nHBC) and BRCA1 and BRCA2 associated HBC exhibit distinct pathobiological features in comparison with nHBC (Marcus et al., 1996 & 1997; Johannsson et al., 1997).

Herein we compare c-erbB2, estrogen receptor (ER) and progesterone receptor (PR) expression in invasive breast carcinomas from HBC patients with known BRCA1 and BRCA2 mutations. We also report the DNA ploidy and S-phase fractions for these neoplasms as determined by flow cytometry. Our findings confirm our previous observations of the pathophenotype of BRCA1 hereditary breast cancers and provide evidence for a distinct, more aggressive pathobiological BRCA2 phenotype compared to previous results.

Methods

Patients. Forty-four patients from BRCA1 HBC families and 26 patients from BRCA2 HBC families meeting study criteria were identified from a larger cohort of HBC patients followed by the hereditary cancer surveillance program headed by Dr. Henry Lynch at the Creighton University School of Medicine. Patients included in the study had a tissue diagnosis of invasive breast carcinoma, known BRCA1 or BRCA2 mutations, and available pathology specimens representative of their primary neoplasm. Informed consent from patients or their survivors and institutional research board approval were obtained prior to the study.

Mutation Analysis. Mutation analysis for BRCA1 and BRCA2 point mutations, insertions and deletions was performed using peripheral lymphocytes as previously described (Puget et al., 1999). Germline BRCA mutation was verified directly in each patient; except for two patients (one BRCA1 and one BRACA2) confirmed to be obligate mutation carriers by demonstration of their respective familial mutations in their offspring.

Immunohistochemistry. Immunostaining was performed on 4.0 micron-thick paraffin-embedded sections heat-fixed on poly-l-lysine-coated glass slides using a Ventana ES automated immunostainer (Ventana Medical Systems, Tucson, AZ). Anti-c-erbB2, a mouse monoclonal antibody (clone CB11) raised against a synthetic peptide corresponding to an internal domain of c-erbB2, was obtained from Ventana. ER- and PR-specific antibodies (clones 6F11& 1A6) were also obtained from Ventana. Only the invasive component of neoplasms were scored for immunoreactivity, *i.e.,* carcinoma *in situ* (CIS) was ignored. C-erbB2 immunostained tumor tissue sections displaying moderate to strong circumferential membrane immunoreactivity were scored as positive. Positive ER and PR immunoreactivity of at least approximately 5% of the neoplastic cell nuclei in the section was scored as positive expression.

Flow Cytometry. For each tumor sample with adequate available tissue two 50 micron-thick sections from formalin-fixed, paraffin-embedded blocks were de-waxed, pepsinized, and stained with propidium iodide using a modified Hedley method (Crissman, et al., 1988). The resultant DNA-stained suspension of extracted nuclei was processed on an EPICS XL-MCL flow cytometer (Coulter Electronics, Hialeah FL), and the accumulated histogram analyzed with MULTICYLE for WINDOWS (Phoenix Flow Systems, San Diego, CA. 1998). DNA histograms were classified and scored with regard to DNA ploidy and percent S-phase fraction, as previously described (Marcus et al., 1996).

Results

c-erbB2 and Steroid Hormone Expression. Paraffin-embedded samples of 44 invasive carcinomas from individuals with BRCA1 mutations and 25 from individuals with BRCA2 mutations were available for

immunostaining. Tumor specimens from 5 out of 44 BRCA1 HBC patients, or 11.4% displayed immunoreactivity indicative of c-erb2b overexpression (Tables 1 & 3). Tweleve out of 25, or 48.0% of specimens from BRCA2 patients showed c-erb2b overexpression (Tables 2 & 3). Tumor cell expression of ER, PR or both was detected in 29.5% (13/44) of BRCA1 patients and 68.0% (17/25) of BRCA2 patients (Tables 1 & 2). Four of 44 (9.1%) of BRCA1 samples and 4 of 25 (16.0%) of BRCA2 samples were ER+ and PR-. No patient samples were PR+ and ER- in either group.

DNA Analysis. Tumor DNA ploidy and percent S-phase fraction analysis also differed between the two groups (Tables 1, 2 & 4). Twenty-five percent of BRCA1 tumors evaluated by flow cytometry (9/36) were diploid and 75.0% (27/36) were aneuploid. The DNA indices of the aneuploid BRCA1 tumors ranged from 1.12 to 2.44 with a mean of 1.64. Six near-tetraploid neoplasms (DNA indices 1.80 to 2.10) were identified. The S-phase fraction ranged from 0.9 % to 5.5 % in diploid BRCA1 tumors and 1.2% to 35.7% in aneuploid BRCA1 tumors. The average S-phase fractions were 2.5% and 13.0% for diploid and aneuploid tumors, respectively.

Among BRCA2 mutation-positive tumors 34.8% (8/23) were diploid and 65.2% (15/23) aneuploid. The DNA index of the aneuploid tumors ranged from 1.07 to 1.94 with a mean of 1.50. Two tumors were near-tetraploid (DNA indices 1.84 to 1.94). The S-phase fractions ranged from 0.4 % to 5.7% for the diploid BRCA2 tumors and 1.8% to 26.0% for the aneuploid BRCA2 breast cancers. The average S-phase fractions were 2.0% for diploid tumors and 7.8% for aneuploid tumors. The average Chi square value for all specimens was 6.1 (range 1.4 to 20.4).

Discussion

The BRCA1 protein is involved in a response to DNA damage and appears to regulate effectors controlling the G2/M cell cycle checkpoint (Yarden et al., 2002) and it is thus not surprising that BRCA1 mutant tumors display a high proliferative index. The function of BRCA2 appears to be to facilitate repair of DNA double stranded breaks and its loss is thought to lead to dependence on less reliable repair mechanisms and chromosome instability (Larminat et al., 2002).

Tumor aneuploidy is generally associated with aggressive neoplasms. The significance of DNA index as a prognosticator of the biological behavior of breast cancer is debatable; however, hypodiploidy and near-tetraploidy appear to be associated with more aggressive breast neoplasms (Hedley et al., 1993). No definitive examples of hypodiploidy could be demonstrated in this series. More of the BRCA1 aneuploid tumors were near-tetraploid (6/27 or 22.2%) than the BRCA2 aneuploid tumors (2/15 or 13.3%). The fact that a high degree of aneuploidy in general is observed in BRCA1 tumors may not contribute to more aggressive biological behavior compared to nHBC, but may simply be a consequence of genomic instability created by loss of G2/M checkpoint control with DNA damage. Consistent with this is the fact that the aneuploid BRCA1 tumors showed high S-phase fractions. High S-phase fraction and negative steroid hormone receptor expression correlate more strongly with aggressive clinical behavior of breast cancer than does DNA ploidy (Hedley et al., 1993; Wegner et al., 1998). These characteristics do suggest that BRCA1 HBC should behave in a more malignant manner than BRCA2 HBC and nHBC. The oncoprotein c-erbB2 is a member of the epidermal growth factor receptor family. Overexpression of c-erbB2 in breast cancers is associated with shortened survival time (Hanna et al., 1994). The relatively low c-erbB2 overexpression rate observed in this series of BRCA1 HBC (11.4 % of cases vs. 16 – 20% of cases in sporadic cancers (Hanna et al., 1994; Hartmann et al., 2001)), however, may partially explain why despite its other aggressive features, BRCA1 is associated with similar survival rates as nHBC (Watson et al., 1998).

Overexpression of c-erbB2 appears to be an independent of estrogen receptor expression as a prognosticator of response to endocrine therapy. Houston et al. (1999) found that c-erbB+ patients had shorter times to progression while on Tamoxifen therapy compared to control patients. The data presented here suggests that a significant percentage of BRCA2 HBC patients may fit in this situation. Adjuvant chemotherapy or immunotherapy with Herceptin^{Tm}, however, may increase the survival of c-erbB2-overexpression positive patients to that comparable to c-erbB2 negative patients. Given the high degree of c-erbB2 expression observed in BRCA2 tumors (Table 3) and otherwise potentially aggressive phenotype of BRCA1 tumors, early establishment of the c-erbB2 status in HBC patients may be even more clinically important than in nHBC in general.

Conclusions

This study confirms previous data showing that BRCA1 HBC displays an aggressive biological phenotype characterized by high S-phase fraction and low estrogen and progesterone receptor expression. C-erbB2 oncoprotein expression was low, however, which may partially explain why BRCA1 HBC tends to have a prognosis no worse than nHBC. BRCA2 cancers showed a slightly lower average S-phase fraction and higher degree of steroid hormone receptor expression than BRCA1 cancers, but also showed a level of c-erbB2 expression about 2.5 times higher than seen in sporadic breast cancers (48% of cases vs. 16 – 20% of cases). This suggests a somewhat more aggressive BRCA2 phenotype than previously thought, as suggested by Agnarsson et al. (1998), and underscores the importance of c-erbB2 testing in HBC as well as nHBC.

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Table 1: DNA Analysis , Steroid Hormone Receptor and c-erbB2 Expression in BRCA1 Mutation Breast Carcinomas. *For hormone receptor and c-erbB2 expression 1 indicates positive and 0 negative immunoreactivity. M denotes male patients and NA indicates data not available.*

Patient	DNA Index	%S-Phase	%Total	X2	ER	PR	c-erbB2	BRCA
1	1.599	35.7	5.2	4.8	0	0	0	I
2	1.552	20.2	25.5	3.9	0	0	0	I
3	1.605	6.5	19.7	8.8	0	0	0	I
4	1	5.5	100	1.9	0	0	0	I
5	1.432	14.5	70.5	7	0	0	0	I
6	1.493	20.3	62.8	1.4	1	0	0	I
7	1.117	18.6	4.4	1.6	0	0	0	I
8	1.638	5.8	77.9	2.2	1	1	0	I
9	1.802	4.3	48.4	3.5	0	0	0	I
10	1	1.5	100	1.6	0	0	0	I
11	1.83	18.9	21.1	3.5	0	0	0	I
12	1.186	4.1	11.8	5.4	1	1	0	I
13	1	1.3	100	13.4	0	0	1	I
14	1	3.4	100	1.7	0	0	0	I
15	2.021	9.6	13.8	11.5	1	1	0	I
16	1.402	7.9	26.4	2.7	0	0	0	I
17	1.68	19.9	13	20.4	0	0	0	I
18	1	1	100	7.6	0	0	0	I
19	NA	NA	NA	NA	1	1	0	I
20	1.672	13.7	10.4	5.5	1	0	0	I
21	1.897	1.2	33.7	14.1	1	1	1	I
22	1.326	27.9	9	7.2	0	0	0	I
23	1.651	24.5	8.4	13.4	0	0	0	I
24	1.897	12.7	49	1.9	0	0	1	I
25	2.097	15.7	10.5	12.3	0	0	0	I
26	1	2.1	100	1.7	0	0	0	I
27	1.135	2	56.4	2	1	1	1	I
28	1.732	10.3	20.4	4.7	0	0	0	I
29	NA	NA	NA	NA	0	0	1	I
30	1.411	9.3	4.9	9.1	0	0	0	I
31	1.586	5.5	28.8	2.1	1	1	0	I
32	2.436	13.3	20.2	2.4	0	0	0	I
33	1.722	14	36	6.5	0	0	0	I
34	1.881	5.8	23.7	2	1	1	0	I
35	1	1	100	11.5	0	0	0	I
36	1	0.9	100	5.7	1	1	0	I
37	1.554	8	32.9	7.4	0	0	0	I
38	NA	NA	NA	NA	1	0	0	I
39	1	5.4	100	3.2	0	0	0	I
40	NA	NA	NA	NA	0	0	0	I
41	NA	NA	NA	NA	0	0	0	I
42	NA	NA	NA	NA	0	0	0	I
43	NA	NA	NA	NA	0	0	0	I
44	NA	NA	NA	NA	1	0	0	I

Table 2: DNA Analysis , Steroid Hormone Receptor and c-erbB2 Expression in BRCA2 Mutation Breast Carcinomas. *For hormone receptor and c-erbB2 expression 1 indicates positive and 0 negative immunoreactivity. M denotes male patients and NA indicates data not available.*

Patient	DNA Index	%S Phase	%Total	X2	ER	PR	c-erbB2	BRCA
1	1.477	6.6	18.6	5.5	0	0	0	2
2	1	3	100	12	NA	NA	NA	2
3	1	0.4	100	1.9	1	1	1	2
4	1.355	26	6.7	4.1	1	0	0	2
5	1	1.3	100	6.4	1	1	0	2
6	1.943	2.2	27	4.7	0	0	0	2
7	1	1.5	100	8.8	1	1	1	2
8	1	5.7	100	3.4	1	0	1	2
9	1.471	9.8	34.6	3.3	0	0	0	2
10	1.698	8.6	40	13.4	1	1	1	2
11	1.116	2.7	19.6	6.1	0	0	1	2
12	1.377	14.9	33.1	19.9	0	0	1	2
13	1.143	1.9	50.6	1.4	1	1	0	2
14	1.841	6.6	63.8	2.7	1	1	0	2
15	1.79	4.3	37.4	3.6	1	1	1	2
16	1	0.7	100	2.3	1	1	1	2
17	1.244	1.8	88.5	3.4	0	0	1	2
18	1.651	4.4	8.6	13.3	1	1	0	2
19	1.599	5.7	23.5	7.8	0	0	1	2
20	1.658	18	3.5	6.4	1	0	1	2
21	1	1.2	100	2.2	1	1	0	2
22	1	2.2	100	3	1	1	1	2
23	1.07	3.5	77.3	6.3	1	1	0	2
24	NA	NA	NA	NA	0	0	0	2
25	NA	NA	NA	NA	1	0	0	2
26	NA	NA	NA	NA	1	1	0	2

Table 3: c-erbB2 and Steroid Hormone Receptor Expression in HBC.

	BRCA1	BRCA2
c-erbB2	5/44 (11.4%)	12/25 (48.0%)
Estrogen Receptor	13/44 (29.5%)	17/25 (68.0%)
Progesterone Receptor	9/44	13/25

Table 4: DNA Ploidy and Percent S-phase Fraction in HBC.

	BRCA1	BRCA2
Diploid	9/36 (25.0%)	8/23 (34.8%)
Diploid %S-phase (mean)	0.9 – 5.5 (2.5)	0.4 – 5.7 (2.0)
Aneuploid	27/36 (75.0%)	15/23 (65.2%)
Aneuploid %S-phase (mean)	1.2 – 35.7 (13.0)	1.8 – 26 .0 (7.8)
DNA Index (mean)	1.12 – 2.44 (1.64)	1.07 – 1.94 (1.50)